To identify the recombinant plasmids, the electrophoretic mobilities of the digestion products of the recombinant DNAs and the non-cloned HPV-DNAs were compared after treatment with a mixture of two restriction endonucleases including the endonuclease used for the insertion of the viral sequences into the plasmid. The number and the sizes of the isolated fragments indicated that in each case the entire viral genomes were integrated. A heterogeneity of the DNA sizes was observed when non-cloned HPV-DNAs or those excised from plasmidic sequences, were analyzed by agarose gel electrophoresis (data not shown). The DNAs from HPVs 14b, 19, 20 and 21 have sizes similar to those of HPVs 3a, 5, 8 and 12 (around 7700 nucleotide pairs) (articles by KREMSDORF (1982) and ORTH (1980) already mentioned).

The sensitivity of cloned viral genomes to 14 restriction endonucleases was analyzed and physical maps established (Figures 1 through 9). The restriction maps of certain HPV-DNAs are repeated in some of the figures for reasons which will be explained further on. Between 22 and 33 cleavage sites have been localized according to the methods previously described (9). No apparent analogy could be detected between the maps with the exception of those of HPVs 14a and 14b, on the one hand (Figures 4a and 4b), and those of HPVs 17a and 17b, on the other (Figure 5). Among the 21 and 31 sites localized respectively on the DNAs of the HPVs 14a and 14b, fifteen turned out to be in common when one of the two BamHI cleavage sites of the DNA of HPV-14a was aligned with the unique BamHI cleavage site of the DNA of HPV-14b. In a similar manner, 21 of the 29 cleavage sites on the DNA of HPV-17a were equally found on the DNA of HPV-17b (out of 26 sites), when the sites of the unique BamHI cleavages wer aligned.

No apparent analogy has been detected between

AS
11/8/04

fragments, the total molecular weight of which equals the size of a genome of the papillomaviruses. The DNA of the new HPV was excised from the DNA of a recombinant bacteriophage, purified by electro-elution, and re-cloned into the plasmid p5P65. A restriction map of the viral DNA was established from the sensitivity of this DNA to 15 restriction endonucleases, which has permitted the localization of 23 cut sites (Figure 9). The map thus established is different from the map of the genomes of HPVs identified up til now. The sequence homology between the DNA of the new HPV and the DNAs of the HPVs identified up to now has been analyzed by molecular hybridization experiments on cultures under strict conditions. A homology, less than 50 %, has been detected between the DNA of the new HPV and the DNA of certain types of HPVs previously identified in the lesions of epidermoplasia verruciformis (HPVs 5, 8, 12, 14, 19, 20, 21 and 25), but no homology was detected with other HPV types. The new virus characterized from an actinic keratosis thus constitutes a new type of HPV provisionally named HPV-IP4.

The use of a radioactive probe prepared from the DNA of purified HPV-IP4 has permitted the demonstration of HPV-IP4 in 42 % of the 17 patients studied having epidermodysplasia verruciformis and in x out of y biopsies of actinic keratosis analyzed. Because of its great frequency among patients of EV, a disease characterized by the frequent development of skin cancers, and because of its association with a fraction of the lesions of actinic keratosis considered as precursors of spinocellular cancers of the skin, HPV-IP4 constitues a type of dermo-tropic HPV with oncogenic potential. It is necessary to incorporate it into any mixture of HPV-DNAs intended for the preparation of molecular probes for the diagnosis or screening of HPV

AJ
11/8/04